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# Environmental Effects of Dredging Technical Notes



## Determining the Developmental Status of Sediment Toxicity Bioassays

### Purpose

This technical note describes events in the generic development of sediment toxicity bioassays for the evaluation of dredged material under section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972 (Public Law 92-532) and section 404(b)(1) of the Federal Water Pollution Control Act of 1972 (Public Law 92-500), as amended. This technical note was written for four reasons:

- To facilitate determining the technical progress of any proposed bioassay by describing its requisite developmental steps.
- To provide the scientific community and regulatory agencies a logical, sequential framework for developing sediment toxicity tests.
- To identify gaps in knowledge and indicate where additional research is needed.
- To suggest a process to the regulatory agencies for evaluating and incorporating a sediment bioassay once it has been accepted by the scientific community.





### Background

Sediment toxicity tests are often conducted in the regulatory evaluation of dredged material. Developing these tests requires research on a variety of topics. Some tests are intuitively more developed and more appropriate for regulatory application than others. However, judging the developmental status of individual tests has been difficult because specific criteria are lacking. This technical note provides initial guidance on this subject by describing the steps taken to develop a sediment toxicity bioassay. However, even technically

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sound sediment toxicity tests may not be appropriate for the regulatory evaluation of dredged material. Again, specific guidance for judging the appropriateness of proposed tests is needed.

## Additional Information

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## Approach

No written guidance exists for judging the developmental status of sediment toxicity tests with regard to the regulatory evaluation of dredged material. For that reason, input was obtained from nearly 40 individuals in the scientific community and regulatory agencies via telephone. Persons contacted represent a geographic balance of the Federal government, private industry and academia (Table 1). Each person was briefed on the purposes of the project, as described above. They were then asked to describe in their own words the characteristics they would expect to see in a fully developed sediment toxicity test intended for the regulatory evaluation of dredged material. Not all persons sought could be reached for comment. For that reason, interested individuals are encouraged to provide written comments to either the author or the EEDP manager.

## Analysis

Results of the telephone survey suggest that most people believe sediment bioassays are developed in an orderly, sequenced fashion. Practitioners know this is not always the case. However, it does suggest that new or proposed tests are judged in a similar fashion by asking the question "How far along in the developmental process is the test?" For that reason, much of the input received during the telephone survey was consolidated into a developmental paradigm for sediment toxicity tests (Phase I). Persons contacted, from both the technical and regulatory communities, strongly indicated that any proposed bioassay must be acceptable to the scientific community. Criteria for judging this acceptance are included in Phase II. Phase III is a description of a process for incorporating a sediment toxicity bioassay into the regulatory evaluation of dredged material after it has been accepted by the scientific community. The above steps are summarized in Table 2.

**Table 1. Persons Contacted in Telephone Survey**

| | |
|---|---|
| W. T. Adams | ABC Laboratories, Columbia, MO |
| R. W. Alden | Old Dominion University, Norfolk, VA |
| D. D. Anderson | U.S. Army Corps of Engineers, St. Paul District, St. Paul, MN |
| G. T. Ankley | U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN |
| S. M. Bay | Southern California Coastal Water Research Project, Long Beach, CA |
| G. A. Burton | Wright State University, Dayton, OH |
| D. J. Call | University of Wisconsin-Superior, Superior, WI |
| E. Casillas | NOAA, National Marine Fisheries Service, Seattle, WA |
| P. M. Chapman | EVS Consultants, Ltd., North Vancouver, BC |
| D. C. Cowgill | U.S. Environmental Protection Agency, Great Lakes National Program Office, Chicago, IL |
| P. A. Dinnell | University of Washington, Seattle, WA |
| J. L. Dorkin | U.S. Environmental Protection Agency, Region V, Chicago, IL |
| T. Fredette | U.S. Army Corps of Engineers, New England Division, Waltham, MA |
| L. Glenbowski | U.S. Army Corps of Engineers, New Orleans District, New Orleans, LA |
| J. F. Hall | Texaco, Inc., Port Arthur, TX |
| D. J. Hansen | U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, RI |
| K. B. Hollar | U.S. Environmental Protection Agency, Region VI, Dallas, TX |
| C. G. Ingersoll | U.S. Fish and Wildlife Service, National Fisheries Contaminant Research Laboratory, Columbia, MO |
| D. R. Kendall | U.S. Army Corps of Engineers, Seattle District, Seattle, WA |
| J. O. Lamberson | U.S. Environmental Protection Agency, Environmental Research Laboratory, Newport, OR |
| J. M. Lazorchak | U.S. Environmental Protection Agency, Cincinnati, OH |
| S. K. Lemlich | U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS |
| J. A. Miller | U.S. Army Corps of Engineers, North Central Division, Chicago, IL |
| D. Nacci | Science Applications International Corporation, Narragansett, RI |
| M. K. Nelson | U.S. Fish and Wildlife Service, National Fisheries Contaminant Research Laboratory, Columbia, MO |
| P. S. Oshida | U.S. Environmental Protection Agency, Region IX, San Francisco, CA |
| W. H. Peltier | U.S. Environmental Protection Agency, Region IV, Athens, GA |
| R. J. Pennington | U.S. Army Corps of Engineers, Jacksonville District, Jacksonville, FL |
| S. I. Rees | U.S. Army Corps of Engineers, Mobile District, Mobile, AL |
| J. R. Reese | U.S. Army Corps of Engineers, North Pacific Division, Portland, OR |
| B. Ross | U.S. Environmental Protection Agency, Region IX, San Francisco, CA |
| N. I. Rubinstein | U.S. Environmental Protection Agency, Environmental Research Laboratory, Narragansett, RI |
| K. J. Scott | Science Applications International Corporation, Narragansett, RI |
| J. D. Smith | U.S. Environmental Protection Agency, Region X, Seattle, WA |
| J. F. Tavolaro | U.S. Army Corps of Engineers, New York District, New York, NY |
| M. L. Tuchman | U.S. Environmental Protection Agency, Region V, Chicago, IL |
| F. J. Urabeck | U.S. Army Corps of Engineers, Seattle District, Seattle, WA |
| C. I. Weber | U.S. Environmental Protection Agency, Cincinnati, OH |
| J. Q. Word | Battelle Northwest Pacific Laboratory, Sequim, WA |

**Table 2. Milestones in the Technical Development and Regulatory Adoption of Dredged Material Toxicity Bioassays**

Phase I — Developmental Paradigm for Sediment Toxicity Bioassays

- Present rationale for developing the bioassay.
- Select appropriate test species.
- Select biological test endpoint(s).
- Characterize contaminant dose-response.
- Develop test procedure.
- Construct statistical design.
- Specify quality assurance/quality control.
- Evaluate test "ruggedness."
- Generate interpretive guidance.
- Conduct bioassay with range of dredged material.

Phase II — Evaluation by the Scientific Community

- Peer-reviewed publications.
- Interlaboratory evaluations.
- Intertest comparisons.
- Acceptance by the scientific community.

Phase III — Evaluation by Federal Regulatory Agencies

- Joint EPA/Corps committee evaluation.
- Training with detailed written protocol.
- Round-robin testing by contract laboratories.
- Joint EPA/Corps committee approval.

## Phase I — Development of the Test Method

### Present Rationale for Developing the Bioassay

The test proponent must clearly depict how the sediment bioassay will be used in the regulatory evaluation of dredged material. Obviously, this requires some knowledge on the part of the test proponent of the regulatory *milieu*. This knowledge should be acquired before test development. Otherwise, considerable resources may be expended in developing a test for which there is no practical use. For example, is the bioassay intended to evaluate bedded or suspended sediments? Is it designed for early tier screening or later evaluations? Is it designed to help implement section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972 (Public Law 92-532) or section 404(b)(1) of the Federal Water Pollution Control Act of 1972 (Public Law 92-500), as amended? Can it be performed by the contracting community or is it restricted to research and development laboratories? Is the cost of the

proposed test in line with current bioassays or would it be expensive to run and require a considerable capital outlay?

### Select Appropriate Test Species

Selection of an appropriate test species is the second and arguably the most critical step in developing a sediment bioassay. Its importance is derived from the fact that biological response is used to "assay" the toxicity of sediment-associated contaminants in dredged material. This biological response, in effect, becomes a "toxicity meter." The following selection criteria must be met:

- *Compatible with test media.* Infaunal species (tube-building or free-burrowing) are used to evaluate bedded sediments while epibenthic, planktonic, or nektonic species are used with suspended sediments.
- *Ecologically, commercially, recreationally important or indigenous.* The biology and natural history of the test species must be documented. For example, what is its ecological function with regard to carbon flow and nutrient cycling.
- *Available throughout the year.* Sufficient numbers of healthy test organisms must be readily available throughout the year either through laboratory cultures or field collections. If cultured, there must be performance criteria for assessing the culture's viability and a published standard operating procedure (SOP) for culturing. If field collected, there must be an acclimation SOP and the effect of seasonality on bioassay results must be documented. For example, what is the seasonal influence of gametic cycle, ambient temperature, recent food availability, and water quality?
- *"Handleable."* Good survival in the negative control treatment and consistent response in the positive control must be achievable on a routine basis by contract laboratories.
- *Documented contaminant sensitivity.* The sensitivity to major classes of contaminants must be documented; details are provided below.

### Select Biological Test Endpoint(s)

Sediment toxicity tests have traditionally measured survival as the primary test endpoint. While this will always continue to be true, a new generation of sediment bioassays that examine sublethal endpoints is now being developed (Dillon in press). These tests typically involve longer (chronic) sediment exposures. The potential number of sublethal endpoints is virtually infinite and includes responses at all levels of biological organization (biochemical, cellular, organismic, population, and community). However, the practical number of sublethal endpoints is much smaller because they must be ecologically relevant, not too difficult to measure, and easily understood outside the scientific community. Reproduction and growth are often cited as two highly desirable sublethal test endpoints (Dillon, Gibson, and Moore 1990). The type of bioassay test endpoint has a major impact on the type of interpretive guidance required (see below).

## Characterize Contaminant Dose-Response

A fundamental principle in toxicology is that no chemical is either inherently toxic or inherently safe. Rather, it is the amount or internal *dose* experienced by the biological receptor that renders a substance toxic or therapeutic (Klaassen, Amdur, and Doull 1986). The quantitative relationship between internal dose and the response that dose elicits is called the dose-response curve. This curve was borrowed early in the formative years of aquatic toxicology to assess the relative toxicities of environmental contaminants (for example, see Sprague 1969). However, it has been used in a significantly different manner. Chemical dose was replaced by external exposure concentration. In other words, the exposure concentration became a *surrogate* for internal dose (Connolly 1985).

One of the many uses of the exposure-response curve in aquatic toxicology was to seek the "most sensitive species." The results of this search have been equivocal. Reviews of aquatic toxicity data (Klapow and Lewis 1979, Thurston and others 1985, Mayer and Ellersieck 1986, and Slooff, van Oers, and de Zwart 1986) as well as convincing theoretical arguments (Cairns and Niederlehner 1987) suggest that seeking a "most sensitive species" may be much like the quest for the "holy grail."

For dredged material bioassays, seeking the "most sensitive species" is even more problematic because sediments are *mixtures* of chemicals. Some of these chemicals are identified by laboratory analysis, but many more are present but never analyzed. The mixture problem is confounded by the fact that these chemicals are embedded in a very complex, heterogeneous geological matrix. Contaminant bioavailability and in situ exposures are affected by these characteristics in a manner not easily understood. For these reasons, dredged material evaluations use "effects-based" testing, that is, allowing the biological response of the test species to integrate the availability and toxicity of all sediment-associated contaminants. Clearly, identifying the "most sensitive species" under these conditions would be quite difficult. Rather, the goal should be to *characterize* the causal relationship between test species' response and major classes of contaminants (for example, metals, chlorinated hydrocarbons, low- and high-molecular weight petroleum hydrocarbons, and pesticides). In a return to fundamental toxicological principles, this characterization should be based on internal *dose* rather than external concentration (Connolly 1985).

## Develop Test Procedure

The experimental protocol is a detailed description of how the proposed test will be conducted. It includes but is not limited to:

- Treatment of sediment before, during, and after the test.
- Treatment of test organism before, during, and after the bioassay.
- Physical conditions (for example, temperature, photoperiod, and aeration).
- Replicate description (for example, size and animals/replicate).

- Feeding.
- Daily activities (for example, visual observations and water quality).
- Duration of test.
- Test termination procedures.
- Measurement of test endpoint.

### Construct Statistical Design

Statistical design is the a priori description of what types and amounts of data are required to adequately test a given hypothesis and how these data will be analyzed. It includes but is not limited to:

- Hypothesis formulation.
- Level of statistical significance.
- Randomization procedures.
- Number of treatments.
- Number of replicates per treatment.
- Population sampling.
- Hypothesis testing (data reduction/data analysis).
- Power analysis.
- Sensitivity analysis.

### Specify Quality Assurance (QA)/Quality Control (QC)

QA/QC is the administrative and technical steps taken to ensure reliable data are produced with specified precision and accuracy. It includes but is not limited to:

- Analysis of intratest variability.
- Analysis of variability at different levels of biological response.
- Acceptable response in negative controls.
- Consistent response in positive controls.
- Development of performance criteria.
- Use of control charts.

### Evaluate Test "Ruggedness"

The American Society for Testing and Materials (ASTM) (1992a) defines "ruggedness" as the "insensitivity of a test method to departures from specified test or environmental conditions." Some of these conditions are identified when the initial test procedure is developed. However, others deal with the intrinsic properties of the sediments and require additional study. Examples include the effects of grain size, interstitial ammonia and sulfides, presence of indigenous fauna, and organic carbon. It has been shown that these factors can

and do bias results of acute lethality sediment bioassays (DeWitt, Ditsworth, and Swartz 1988, and Ankley, Katko, and Arthur 1990). Their potential influence will no doubt increase when test duration increases and more sensitive endpoints are examined (that is, chronic sublethal sediment bioassays). It is therefore incumbent upon the test proponent to evaluate these factors. Guidance for evaluating test "ruggedness" has been provided by ASTM (1989). Results should be summarized as a matrix of conditions under which the test should or should not be conducted.

### Generate Interpretive Guidance

The bioassay proponent must provide the technical basis for interpreting the biological and ecological importance of test results. Interpretive guidance should not be confused with statistical significance. The latter is an arbitrary (but hopefully not capricious) means of judging numerical data within a specific level of confidence. Interpretive guidance, on the other hand, explains the biological importance of the observed results. For example, if a project sediment causes a statistically significant 5 percent decrease in survival or growth, is that truly detrimental to the organism? Would a 10 percent decrease be twice as "bad" or only incrementally injurious? A more concrete example can be found in contemporary sediment bioassays conducted with two of the most commonly used species — *Rhepoxynius abronius* and *Ampelisca abdita*. An observation of 30 percent mortality in *R. abronius* is probably much worse than 30 percent mortality in *A. abdita* simply because the former is an annual species and the latter has multiple broods per season. Generating interpretive guidance for sublethal endpoints represents an even greater challenge than that required for survival data.

### Conduct Bioassay with Range of Dredged Material

Once a draft protocol has been developed, the test should be conducted on a range of well characterized sediments representing suspected low and high toxicity. Gauging the success (or failure) of this initial sediment testing will be directly dependent on the preceding research and development. If sufficient time and effort has been devoted to the issues described in Phase I above, this initial foray with natural sediments should result in only minor adjustments to the protocol. Too many sediment bioassays probably enter this phase prematurely.

## Phase II — Evaluation by the Scientific Community

### Peer-Reviewed Publications

The test proponent must communicate the research results in peer-reviewed publications. This activity serves several functions. First, it permits simultaneous access to the test protocol to everyone in the scientific community. This examination promotes and focuses scientific debate. Before publication, knowledge is anecdotal and typically limited to informal communications between

colleagues. Acceptance for peer-review publication, however, does not necessarily imply endorsement nor acceptance on the part of the scientific community. In fact, some editors will publish marginal manuscripts in an effort to induce scientific debate.

Second, increased scrutiny brought on as a result of peer-review publication will greatly increase the probability that weakness in a proposed test method will be discovered — a healthy process. Exposing weaknesses does not necessarily disqualify any bioassay. On the contrary, it usually leads to significant improvements. At the very least, it helps define the test's limits of applicability.

Third, in a good, well written journal article, the author will identify knowledge gaps and recommend important areas for further research and development. At this point in its development, the proposed sediment bioassay is beginning to move out of its laboratory of origin and into the larger family of research laboratories.

### Interlaboratory Evaluations

If there are sufficient resources and technical interest, the proposed method will be conducted by other research and development laboratories. This is an important and critical step in the evolution of any test method. Interlaboratory evaluations can be designed to accomplish one or more goals.

- Improve specific aspects of the test method via targeted research.
- Expand the domain of bioassay response with other dredged material.
- Evaluate interlaboratory variation.
- Compare response with other sediment bioassays (see below).

### Intertest Comparisons

Once an initial draft protocol has been modified and refined through debate and research in the scientific community, it is ready for comparison to other sediment bioassays. For this comparison to be meaningful, it must be conducted in an equitable fashion; that is, same sediment, same time, same place, same temperature, and so forth. Intertest comparisons under dissimilar circumstances are not valid. One purpose of the intertest study is to examine how frequently and with what precision a particular bioassay indicates toxicity relative to other sediment bioassays. It is *not* designed to identify the "most sensitive bioassay." As with species sensitivity, finding the single most sensitive sediment bioassay is probably not achievable. Most intertest studies recognize this fact and recommend using a battery of sediment bioassays (Burton and others 1989, Giesy and Hoke 1989, Long and Buckman 1989, and Pastorok and Becker 1990).

### Acceptance by the Scientific Community

The scientific community has developed little written guidance for accepting or rejecting individual bioassays. Instead, a "survival of the fittest" process usually takes place. Over time, some bioassays are examined and used with greater frequency, while others receive less and less attention. Eventually, some tests disappear from laboratory evaluation altogether. This is usually a slow but healthy process. Close scrutiny by many investigators ensures "survival of the fittest"; that is, tests that work and are biologically meaningful. If this process has one weakness, it is determining precisely when a particular test has been accepted (or rejected) by the scientific community. Many of those contacted during the telephone survey indicated that being able to discern when the scientific community had made this judgment was very important to them.

Probably the most discrete temporal event connoting scientific acceptance of a sediment bioassay is publication by ASTM's Subcommittee E47.03 on Sediment Toxicity. However, the reader should realize that even these ASTM documents are not step-by-step "cookbooks." In ASTM parlance, these reports are *guides* — "a series of options or instructions that do not recommend a specific course of action" (ASTM 1992b). The lack of an instruction manual does not mean that the Subcommittee members cannot make a decision. Rather, it reflects the true state-of-the-practice in sediment toxicity testing.

## Phase III — Evaluation by Federal Regulatory Agencies

### Joint EPA/Corps Committee Evaluation

Open-water disposal of dredged material is evaluated under regulations implementing portions of two laws: section 103 of the Marine Protection, Research, and Sanctuaries Act of 1972 (Public Law 92-532) and section 404(b)(1) of the Federal Water Pollution Control Act of 1972 (Public Law 92-500), as amended. Joint Federal regulatory responsibility is vested with the U.S. Army Corps of Engineers and the U.S. Environmental Protection Agency. These agencies have created two permanent joint committees to oversee the technical implementation of these laws and regulations. Because the regulations include sediment bioassays in the evaluation of dredged material, it is logical that these two committees review and judge the appropriateness and acceptability of proposed sediment bioassays.

The basis for evaluating a sediment bioassay is much broader than just technical soundness. As public servants and custodians of the public welfare, regulatory agencies are required to balance resource expenditures with benefits received in all Federal actions. They must be able to explain to the public or, in the case of permitted activities, to the private sector, precisely why the test is being conducted, what information it will yield, and how that information will be used in decision-making. Important criteria used by regulatory agencies in evaluating a sediment bioassay include but are not limited to:

- Relevant and appropriate for the intended use.
- Founded in the applicable laws and regulations.
- Accepted by the scientific community.
- Accompanied by interpretive guidance.
- Demonstrated track record with a variety of dredged material.
- Cost-effective.
- Able to sustain judicial review.
- Simplified "cookbook" version of the bioassay available.
- "Doable" in a routine fashion by contract laboratories.

### Training with Detailed Written Protocol

Once a technically sound method has been developed and accepted by the scientific community, some level of training is highly desirable. Accompanying this training should be a simplified step-by-step instruction manual. This instruction manual should be based on the appropriate detailed technical documentation, but should not include extraneous material not required for conducting the bioassay in a technically sound manner.

### Round-robin Testing with Contract Laboratories

Contract laboratory performance is analyzed by round-robin testing. The purpose is to evaluate the laboratories' technical ability to conduct the test, establish market-based costs for conducting the bioassay, determine interlaboratory variability, and expand the track record for this bioassay with a greater variety of dredged material. Use of these round-robin data to determine the acceptability of specific project materials will be made on a case-by-case basis.

### Joint EPA/Corps Committee Approval

Once the above steps have been completed, the EPA/Corps joint technical committees should formally approve (or disapprove) a particular sediment toxicity test.

## Sediment Bioaccumulation Bioassays

The focus of this technical note was on the technical development and potential regulatory use of sediment toxicity bioassays. The same approach can be applied to sediment bioaccumulation bioassays. In that case, many of the Phase I elements (test development) would be different. However, much of Phase II and Phase III activities (evaluation by the scientific and regulatory communities, respectively) would be very similar.

## Future Activities

This technical note provides initial guidance for determining the developmental status of sediment toxicity tests for the regulatory evaluation of dredged material. It will form the basis for a workshop to be conducted in FY 93. The purpose of the workshop will be to comment on the content and completeness of this technical note. Participants will be charged with prioritizing developmental milestones and assigning attributes such as "must," "should," and "could" to each milestone. Invited participants will be those who are actively involved in developing and regulating with dredged material toxicity bioassays. Following the workshop, final guidance will be published as a technical note.

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